In re Appln. of Kovesdi et al.
Application No. Not Assigned (Continuation of U.S. Patent App. No. 08/258,416)

45. (New) A method for the production of a recombinant adenovirus which is defective at least for the E4 region, comprising infecting the cell line of claim 36 with the E4 defective adenovirus and harvesting the adenovirus.

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46. (New) The method according to claim 45, wherein the cell line cells are transformed with one or more plasmids providing the various regions of the genome of the defective recombinant adenovirus.

47. (New) The method according to claim 46, wherein the recombinant adenovirus is defective for E1 and E4 regions.

Sub C2 48. (New) A defective recombinant ~~adenovirus ΔE1, ΔE4, wherein all~~ or part of the E1 region ~~and the~~ whole of the E4 region is deleted.

REMARKS

Amendments to the Specification

The specification has been amended to correct inadvertent simple typographical errors identified during the prosecution of the parent application.

Interfering Claims

The text of new claims 36-47 has been copied from U.S. Patent 6,127,175 (Vigne et al.) for the purpose of provoking an interference with claims 1, 3, 4, 5, 11, 12, 15, 16, 20, 23, 24, and 25 of the Vigne '175 patent. Claim 48 of the present application is substantially identical to claim 33 of the Vigne '175 patent, such that an interference between these claims also is appropriate (see, e.g., M.P.E.P. § 2306). The Vigne '175 patent issued on October 6, 2000, which is less than one year prior to the date of this Preliminary Amendment. Thus, the claims presented herein are admissible under 35 U.S.C. § 135. Applicants submit herewith a Request for Declaration of Interference identifying proposed counts and providing all

additional required information. Briefly, claims 36-48 are patentable over the Vigne '175 patent inasmuch as the effective filing date of the subject patent application precedes the effective invention date attributable the Vigne '175 patent. The reasons for Applicants' entitlement to a judgment of priority in the proposed interference are further described in the Request for Declaration of Interference.

Discussion of Claim Amendments

Claims 36-48 are supported by the originally filed specification. Specifically, claims 36-44 find support at, e.g., page 29, line 36 - page 30, line 25 (Example 9), and claims 45-48 find support at, e.g., page 24, line 23 - page 25, line 23 (Example 3). As such, new claims 36-48 add no new matter.

Conclusion

The application is considered in good and proper form for allowance, and the Examiner is respectfully requested to process this application for interference proceedings. If, in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned attorney.

Respectfully submitted,

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PATENT
Attorney Docket No. 213257

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Kovesdi et al.

Application No.: Not Assigned
(Continuation of U.S. Patent App. No. 08/258,416)

Filed: September 19, 2001

For: COMPLEMENTARY ADENOVIRAL VECTOR SYSTEMS AND CELL LINES

Art Unit: Not Assigned

Examiner: Not Assigned

AMENDMENTS TO THE SPECIFICATION MADE VIA PRELIMINARY AMENDMENT

Page 6, line 29, through page 7, line 20, has been amended to read as follows:

Assembly of the virion is an intricate process from the first step of assembling major structural units from individual polypeptide chains (reviewed in Philipson, "Adenovirus Assembly," *In The Adenoviruses,* Ginsberg, ed., Plenum Press, New York, NY (1984), pp. 309-337; Horwitz (1990), *supra*). Hexon, penton base, and fiber assemble into trimeric homopolymer forms after synthesis in the cytoplasm. The 100 kd protein appears to function as a scaffolding protein for hexon trimerization and the resulting hexon trimer is called a hexon capsomere [capsomer]. The hexon capsomeres can self-assemble to form the shell of an empty capsid, and the penton base and fiber trimers can combine to form the penton when the components are inside the nucleus. The facet of the icosahedron is made up of three hexon capsomeres, which can be seen by dissociation of the capsid, but the intermediate step of formation of a group-of-nine hexons has not been observed. Several assembly intermediates have been shown from experiments with temperature-sensitive mutants. The progression of capsid assembly appears dependent on scaffolding proteins, 50 kd and 30 kd, and the naked DNA most probably enters the near-completed capsid through an opening at one of the vertices. The last step of the process involves the proteolytic trimming of the precursor polypeptides pVI, pVII, pVIII and pTP, which

stabilizes the capsid structure, renders the DNA insensitive to nuclease treatment, and yields a mature virion.

Page 20, lines 13-14, has been amended to read as follows:

This example describes the generation of one embodiment involving $\underline{Ad_{GV}.10}$ [AD_{GV}.10], namely Ad_{GV}CFTR.10.

Page 25, line 29, through page 26, line 13, has been amended to read as follows:

Ad_{GV}.13 is characterized by not only complete elimination of E1, and E4 (as in $\underline{Ad_{GV}.12}$ [AD_{GV}.12]) but also complete elimination of E2A. The complete coding region of E2A is deleted by fusing together the DNA from two E2A mutant viruses, namely H5in800 and H5in804, containing insertions of *Cla* I restriction sites at both ends of the open reading frame (Vos et al., *Virology*, *172*, 634-642 (1989); Brough et al., *Virology*, *190*, 624-634 (1992)). The *Cla* I site of H5in800 is between codons 2 and 3 of the gene, and the *Cla* I site of H5in804 is within the stop codon of the E2A gene. The resultant virus contains an open reading frame consisting of 23 amino acids that have no similarity to the E2A reading frame. More importantly, this cassette offers yet another region of the virus genome into which a unique gene can be introduced. This can be done by inserting the gene of interest into the proper reading frame of the existing mini-ORF or by introducing yet another expression cassette containing its own promoter sequences, polyadenylation signals, and stop sequences in addition to the gene of interest.

Page 30, lines 27-29, has been amended to read as follows:

This cell line is suitable for complementing vectors that are deficient in the E1 and E4 <u>regions</u> [region], such as the Ad_{GV}CFTR.12 series of vectors.

Page 30, line 35, through page 31, line 18, has been amended to read as follows:

The E2A expression cassette for introduction into 293/E4 cells is produced as follows. The first step is to alter surrounding bases of the ATG of E2A to make a perfect Kozak consensus (Kozak, *J. Molec. Biol.*, *196*, 947-950 (1987)) to optimize expression of E2A. Two primers are designed to alter the 5′ region of the E2A gene. Ad5s(23884), an 18 bp oligonucleotide

(5′GCCGCCTCATCCGCTTTT3′ [5′gCCgCCTCATCCgCTTTT3′]) (SEQ ID NO:3), is designed to prime the internal region flanking the *Sma* I site of the E2A gene. DBP(ATG)R1, a 32 bp oligonucleotide (5′CCGGAATTCCACCATGGCGAGTCGGGAAGAGG3′ [5′CCggAATTCCACCATggCgAgtcgggAAgAgg3′]) (SEQ ID NO:4), is designed to introduce the translational consensus sequence around the ATG of the E2A gene modifying it into a perfect Kozak extended consensus sequence and to introduce an *Eco* RI site just 5′ to facilitate cloning. The resulting PCR product using the above primers is digested with *Eco* RI and *Sma* I (NEB) and cloned into the identical polylinker sites of pBluescript IIKS+ (Stratagene [Stratgene], La Jolla, CA). The resulting plasmid is named pKS/ESDBP.

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Art Unit: Not Assigned

Examiner: Not Assigned

AMENDMENTS TO THE CLAIMS
MADE VIA PRELIMINARY AMENDMENT

[1. An adenoviral vector that is deficient in two or more adenoviral gene functions.]

[2. The adenoviral vector of claim 1, wherein at least one of the said two or more gene functions is selected from the group of gene functions comprising the E1, E2, E3 and E4 regions of the adenoviral genome.]

[3. The adenoviral vector of claim 1, wherein at least one of the said two or more gene functions is selected from the group of gene functions comprising the late regions of the adenoviral genome.]

[4. The adenoviral vector of claim 2, wherein at least one of the said two or more gene functions is selected from the group of gene functions comprising the late regions of the adenoviral genome.]

[5. The adenoviral vector of claim 1, wherein the said two or more adenoviral gene functions is all the adenoviral gene functions.]

[6. The adenoviral vector of claim 5, wherein said adenoviral vector comprises adenoviral inverted terminal repeats and one or more adenoviral promoters.]

[7. The adenoviral vector of claim 5, wherein said adenoviral vector comprises adenoviral inverted terminal repeats and a packaging signal.]

[8. The adenoviral vector of claim 1, wherein said adenoviral vector only functions in a complementing cell line.]

[9. The adenoviral vector of claim 8, wherein said adenoviral vector only functions in a complementing cell line as a result of the modification of adnoviral inverted terminal repeats or packaging signal.]

[10. A cell line that complements an adenoviral vector of claim 1.]

[11. A cell line that complements an adenoviral vector of claim 2.]

[12. A cell line that complements an adenoviral vector of claim 3.]

[13. A cell line that complements an adenoviral vector of claim 4.]

[14. A cell line that complements an adenoviral vector of claim 5.]

[15. A cell line that complements an adenoviral vector of claim 6.]

[16. A cell line that complements an adenoviral vector of claim 7.]

[17. A cell line that complements an adenoviral vector of claim 8.]

[18. A cell line that complements an adenoviral vector of claim 9.]

[19. A cell line selected from the group consisting of those cell lines designated as 293/E4, 293/ORF-6, and 293/E4/E2A.]

[20. A recombinant multiply deficient adenoviral vector of claim 1 comprising a foreign gene.]

[21. The recombinant vector of claim 20, wherein said foreign gene is the cystic fibrosis transmembrane regulator gene.]

[22. The recombinant vector of claim 20, wherein said recombinant vector is selected from the group consisting of Ad_{GV}.10, Ad_{GV}.11, Ad_{GV}.12, and Ad_{GV}.13.]

[23. The recombinant vector of claim 22, wherein said recombinant vector is selected from the group consisting of Ad_{GV}CFTR.10, Ad_{GV}CFTR.11, Ad_{GV}CFTR.12, and Ad_{GV}CFTR.13.]

[24. A recombinant multiply deficient adenoviral vector of claim 1 comprising a DNA sequence capable of expressing in a mammal a therapeutic agent.]

[25. The recombinant multiply deficient adenoviral vector of claim 24, wherein said therapeutic agent is an antisense molecule selected from the group consisting of mRNA and a synthetic oligonucleotide.]

[26. A recombinant multiply deficient adenoviral vector of claim 1 comprising a DNA sequence capable of expressing in a mammal a polypeptide capable of eliciting an immune response to said polypeptide.]

[27. A method of gene therapy comprising the administration to a patient in need of gene therapy a therapeutically effective amount of a recombinant multiply deficient adenoviral vector of claim 20.]

[28. A method of gene therapy comprising the administration to a patient in need of gene therapy a therapeutically effective amount of a recombinant multiply deficient adenoviral vector of claim 21.]

[29. A method of gene therapy comprising the administration to a patient in need of gene therapy a therapeutically effective amount of a recombinant multiply deficient adenoviral vector of claim 22.]

[30. A method of gene therapy comprising the administration to a patient in need of gene therapy a therapeutically effective amount of a recombinant multiply deficient adenoviral vector of claim 23.]

[31. The method of claim 28, wherein the recombinant multiply deficient adenoviral vector is administered to the lungs of said patient.]

[32. The method of claim 30, wherein the recombinant multiply deficient adenoviral vector is administered to the lungs of said patient.]

[33. A method of therapy comprising the administration to a patient in need of therapy a therapeutically effective amount of a recombinant multiply deficient adenoviral vector of claim 1 comprising a DNA sequence capable of expressing a therapeutic agent.]

[34. The method of claim 33, wherein said therapeutic agent is an antisense molecule selected from the group consisting of mRNA and a synthetic oligonucleotide.]

[35. A method of vaccination comprising the administration to a patient in need of vaccination an immunity-inducing effective amount of a recombinant multiply deficient adenoviral vector of claim 1 comprising a DNA sequence capable of expressing a polypeptide capable of eliciting an immune response to said polypeptide.]

36. A recombinant cell line for the production of a defective adenovirus, comprising, inserted into its genome, part of an adenovirus E4 region comprising an ORF6 reading frame under the control of a functional promoter, wherein the inserted E4 region does not contain a functional ORF4 reading frame.

37. The cell line according to claim 36, wherein the E4 region is derived from a group C human adenovirus genome.

38. The cell line according to claim 37, wherein the E4 region is derived from the genome of an Ad2 or Ad5 adenovirus.

39. The cell line according to claim 36, wherein the promoter is an inducible promoter.

40. The cell line according to claim 36, which transcomplements for the E1 region.

41. The cell line according to claim 40, which is derived from cell line 293.

42. The cell line according to claim 36, wherein the part of the E4 region does not contain ORF4.

43. The cell line according to claim 42, wherein the part of the E4 region does not contain ORF1-ORF4.

44. A plasmid comprising part of an E4 region of an adenovirus genome carrying a reading frame ORF6 under the control of an inducible promoter.

45. A method for the production of a recombinant adenovirus which is defective at least for the E4 region, comprising infecting the cell line of claim 36 with the E4 defective adenovirus and harvesting the adenovirus.

46. The method according to claim 45, wherein the cell line cells are transformed with one or more plasmids providing the various regions of the genome of the defective recombinant adenovirus.

47. The method according to claim 46, wherein the recombinant adenovirus is defective for E1 and E4 regions.

48. A defective recombinant adenovirus ΔE1, ΔE4, wherein all or part of the E1 region and the whole of the E4 region is deleted.